APPENDIX B REAGENTS BIOMEK®2000 AUTOMATION WORKSTATION PROCEDURES MANUAL - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION IV Page 1 of 3 Issue No. 4 Effective Date: 8-August-2005

APPENDIX B: REAGENTS

1. This appendix describes the preparation of reagents necessary for the DNA analysis. For each reagent listed, the company and catalog number is included. As a reagent is prepared, it will be labeled to include the following information:

Identity
Concentration
Lot number
Date of preparation
Initials of preparer
Date of expiration

and if appropriate:

Date of autoclaving Storage requirements

- 2. All reagents will be prepared with Type I water, unless other wise stated.
- 3. All chemicals and reagents will be stored according to the manufacturers' specifications. All chemicals containing biologicals will be disposed of in biohazard bags. Waste organic reagents will be placed in designated waste containers in a hood and removed from the laboratory by an appropriate means of disposal. All other reagents can be disposed of in the laboratory sink.
- 4. Any changes in chemical supply companies will be carefully checked by the Section Supervisor to ensure the chemical being provided meets the specifications necessary for the reagent. Any changes in chemical supply companies will be brought to the Forensic Biology Section Chief's attention so the list can be updated as necessary.
- 5. Concentrations preceding reagent components reflect the final concentrations of that specific component in the resulting mixture.

ALUQUANT™ HUMAN DNA QUANTITATION KIT

Expiration date: Manufacturer's expiration date.

Purchased from Promega Corporation, Madison, WI., Catalog Number DC1011 for 400 reaction kit.

Store all AluQuantTM Human Quantitation System reagents at -20°C.

Kit components included (expiration date same as kit unless specified):

- ALUQUANTTM ENZYME SOLUTION
- ALUQUANTTM DENATURATION SOLUTION
- ALUQUANT™ NEUTRALIZATION SOLUTION
- ALUQUANTTM PROBE MIX

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• ENLITEN® LUCIFERASE/LUCIFERIN (L/L) REAGENT

Expiration date: AluQuantTM Human Quantitation System kit expiration data when in lyophilized state. Once reconstituted discard after two weeks.

Equilibrate to room temperature prior to use. Add the ENLITEN® Luciferase/Luciferin (L/L) Reconstitution Buffer to the ENLITEN® Luciferase/Luciferin Reagent in the brown bottle and then inverted the bottle several times to mix.

Store unused portions of Enliten® Luciferase/Luciferin (L/L) Reagent in a -20°C up to two weeks.

• ENLITEN® LUCIFERASE/LUCIFERIN (L/L) RECONSTITUTION BUFFER

Equilibrate to room temperature prior to use.

- HUMAN GENOMIC DNA STANDARD (20 ng/μL)
- NUCLEASE-FREE WATER

DITHIOTHREITOL, 0.39 M (DTT)

Expiration date: Six months from date of preparation

MW 25 mL

DTT 154.2 1.50 g Type I Water 15 mL

Add DTT to Sterile Type I Water and mix well. When DTT is completely dissolved, bring up to final volume with Sterile Type I Water. Filter sterilize and divide into $500 \, \mu L$ aliquots in sterile microcentrifuge tubes and store at $-20 \, C$.

DTT (Molecular Biology Grade) - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number D0632, 25 g bottle.

DNA IOTM SYSTEM

Expiration date: Manufacturer's expiration date

Purchased from Promega Corporation, Madison, WI., Catalog Number DC6700 for 400 reaction kit.

Store all DNA IQTM Isolation System reagents at room temperature.

Kit components included (expiration date same as kit unless specified):

• DNA IQ™ LYSIS BUFFER

Expiration date: One month from the date when DTT is added

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Add 2.5 μ L of 0.39 M DTT for every 100 μ L of DNAIQTM Lysis Buffer that is prepared. Mark and date label to record the addition of DTT. This solution can be stored at room temperature for up to a month if capped tightly.

- DNA IQ™ ELUTION BUFFER
- DNA IQTM WASH BUFFER

Add 35 mL of 95% ethanol and 35 mL of Isopropyl Alcohol to 2X Wash Buffer. Be certain to accurately measure the alcohol volumes since this could negatively impact the performance of the robotic extraction. Mix contents and store at room temperature in a tightly capped container.

ETHANOL, 95%

Expiration date: Dispose of when necessary

Purchased as Reagent Alcohol (190 Proof) from VWR, Bridgeport, NJ, Catalog number 6590-1, 4 L bottle.

HYDROCHLORIC ACID (250 mM)

Expiration date: AluQuantTM Human Quantitation System kit expiration data

Dilute the HCl reagent from 250 mM to 200 mM prior to use. After vortexing or swirling to mix the HCl reagent, remove 12 mL of HCl reagent using a pipette and transfer to a clean 15 mL conical tube. Add 3 mL of sterile Type I water and mix by inverting several times. Label the tube "200 mM HCl", date and initial it. It can be stored at room temperature at the robot workstation.

The HCl Acid (250 mM) Solution is supplied as a component in the AluQuantTM Human Quantitation System kit

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